Neuronal Nicotinic Receptor Subtypes

JON LINDSTROM, RENÉ ANAND, XIAO PENG,
VOLODYMYR GERZANICH, FAN WANG,
AND YUEBING LI

Department of Neuroscience
Medical School of the University of Pennsylvania
Philadelphia, Pennsylvania 19104-6074

Nicotinic acetylcholine receptors (AChRs) are members of a gene superfamily of ligand-gated ion channels that includes $GABA_A$ receptors, glycine receptors, and 5HT-3 serotonin receptors,[1,2] but does not appear to include glutamate receptors.[3] AChRs are the predominant excitatory receptors in the mammalian peripheral nervous system, whereas glutamate receptors are the predominant excitatory receptors in the central nervous system. In the peripheral nervous system AChRs are critical for controlling skeletal muscles and consequently are the target of toxins such as snake venom toxins like α-bungarotoxin (αBgt), and muscle AChRs are the target of an antibody-mediated autoimmune response in myasthenia gravis.[4] Although outnumbered by glutamate receptors in the vertebrate central nervous system, neuronal AChRs are widespread. Their functional roles are not yet well known. They have been implicated in learning and memory, and their number is decreased in Parkinson's syndrome and Alzheimer's disease.[5] One measure of their significance is that through nicotine they mediate the addiction to tobacco, which is predicted to cause a quarter of a billion premature deaths by the turn of the century.[6]

The three branches of the AChR gene family[7] are (1) muscle AChRs, (2) neuronal AChRs that, unlike those of muscle, do not bind αBgt, and (3) neuronal AChRs that do bind αBgt. All are ACh-gated cation channels formed probably from five subunits of usually two to four homologous types.[8,9] These subunits are thought to be organized like barrel staves around a central cation channel. The synthesis, structure, and function of muscle-type AChRs are known in relatively great detail, whereas the more diverse structures, functional properties, and functional roles of neuronal AChRs are much less well characterized.[7–9] The functional properties of various combinations of neuronal AChR subunits expressed in *Xenopus* oocytes have been better characterized than have the functional properties of diverse neuronal AChR subtypes *in vivo,* and the actual functional roles in the nervous system of many of the real and potential subtypes of neuronal AChRs remain to be determined. It is becoming apparent that many neuronal AChRs are likely to have functional roles that differ from the straightforward postsynaptic type of critical link in neurotransmission exemplified by muscle AChRs.

We attempt to review briefly the diversity of neuronal AChRs primarily from the perspective of what has been done in our laboratory. Muscle AChRs, which serve as an archetype for the gene family, are considered only briefly as a model for comparison with neuronal AChRs. Most emphasis is given to neuronal AChRs that bind αBgt, because they have been the most recent focus of research attention in this field. Data are presented that compare and contrast some of the properties of representatives of each of the three branches of the AChR gene family as revealed by expressing their cRNAs in *Xenopus* oocytes.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

MUSCLE AChRs

Schematic representations of the known AChR subunit sequences are depicted in FIGURE 1. All AChR subunits consist of homologous sequences[7–9] that start with a signal sequence cleaved during translation. The N-terminal ≈200 amino acids of the mature subunits are thought to form a large domain on the extracellular surface. This is followed by ≈90 amino acids comprising three closely spaced hydrophobic sequences that are thought to form transmembrane domains (M1—M3). M2 is thought to line the ion channel. Following M3 is a large cytoplasmic domain of ≈100–200 amino acids. The large cytoplasmic domain is the most variable part of the sequence between subunits. In many AChR subunits this region contains the most immunogenic epitopes that are recognized by antibodies in both native and denatured subunits.[10] Thus, antibodies raised against bacterially expressed peptides from this region have been very effective at recognizing both native and denatured AChRs.[11,12] The large cytoplasmic domain is followed by a fourth hydrophobic sequence (M4) leading to a small ≈10–30 amino acid extracellular domain at the C-terminus. Among the methods that have been applied to determining the transmembrane orientation of the AChR subunit polypeptide chains is the "reporter epitope" method.[13] In this method, epitope sequences are inserted by *in vitro* mutagenesis, and then the transmembrane orientation of the tagged part of the sequence is determined using a monoclonal antibody (mAb) to the epitope to label the extracellular or cytoplasmic surface of the AChR expressed from cRNA in *Xenopus* oocytes. The reporter epitope method has the technical virtue that it avoids the slow and expensive process of making new antibodies. It should be able to probe the transmembrane orientation of any part of a subunit sequence that is on the protein surface and that is not part of an active site. The basic method should be applicable to any cloned membrane protein.

AChR subunit genes are scattered over several human chromosomes: 1(β2), 2(α1,γ,δ), 8(α2), 15(α3, α5, α7, β4), 17(β1), and 20(α4).[14–19] Genes for subunits that are components of a native AChR are not necessarily located together. Thus, the genes for α1, β1, γ, and δ subunits of muscle AChR are on two chromosomes, as is the case for the genes for α4 and β2 subunits of the brain AChR subtype with high affinity for nicotine. However, the α3, β4, and α5 genes that encode subunits of ganglionic AChRs are contiguous on a single chromosome.

Muscle α1 subunits mature in their conformation after synthesis and before assembly with other subunits.[20] This conformational maturation is marked by their acquisition of the ability to bind αBgt to what will become the ACh binding site (and is in part composed of amino acids in the region 180–200)[8] and mAbs to the main immunogenic region (which is in part composed of amino acids in the region 66–76).[21,22] This conformational maturation is thought to be associated with formation of a disulfide bond between cysteines α128 and α142[23] to form a loop which is conserved in all AChR subunits, and which in most contains an N-glycosylation site at 141.[7–9] Similar maturation events may occur with all AChR subunits, but have not been studied. Ability to bind ACh and small cholinergic ligands is not acquired until α1 associates with γ, δ, or ϵ subunits; the binding site is thought to be formed at the interface between subunits.[24–26] The extracellular domain is thought to be largely responsible for the specific associations between muscle AChR subunits.[25] Sequence homologies suggest that similar considerations apply to the assembly of neuronal AChR subunits and the formation of ACh binding sites at some subunit interfaces.

At mature neuromuscular junctions the AChRs are composed of two α1 subunits in combination with one each of β1, δ, and ϵ subunits.[8] Extrajunctional AChRs found

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

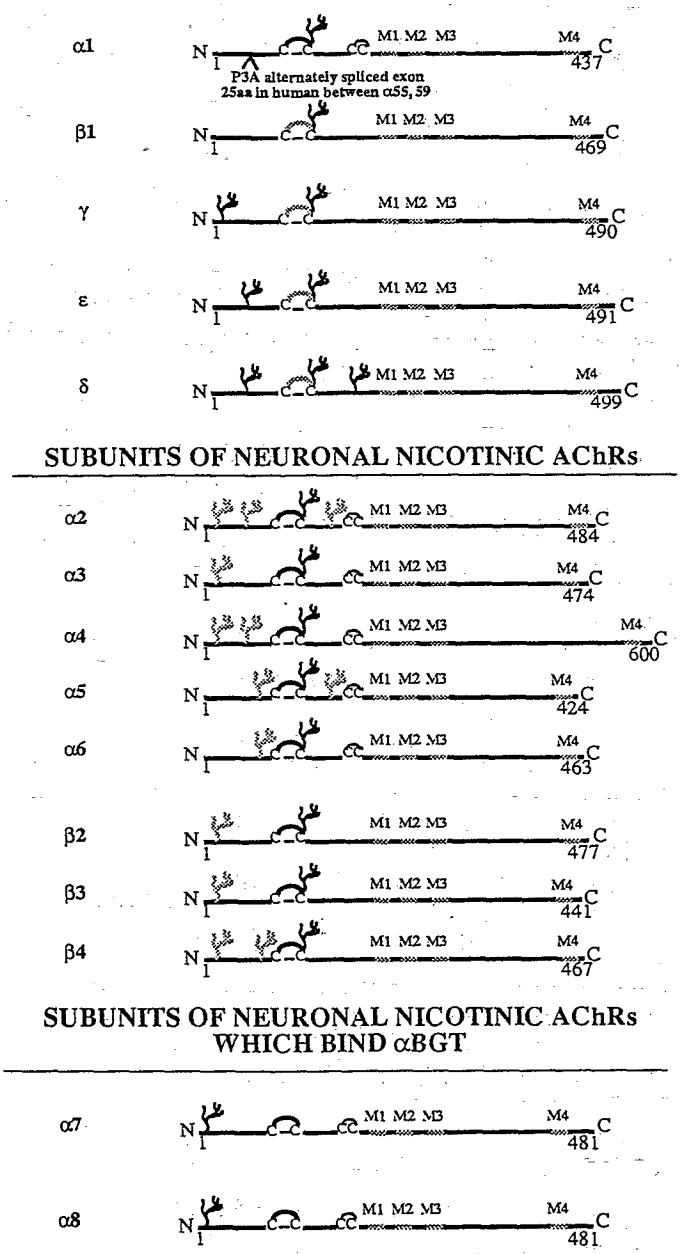

FIGURE 1. Comparison of AChR subunit sequences. The sequences of known AChR subunits are schematically represented to indicate the homologies in their structures. The complete aligned amino acid sequences of all rat AChR subunits are listed in reference 7.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

before innervation or after denervation differ from mature AChRs by substituting γ for ε subunits, resulting in increased duration of channel opening and an increased rate of turnover, among other changes. The subunits are thought to be arranged around a central cation channel in the order α1, δ, α1, ε, β in such a way that two acetylcholine binding sites are formed, one at the interface between α and δ subunits and the other between α1 and ε subunits. Evidence suggests that the channel of both muscle and neuronal AChRs is lined by amino acids from the second transmembrane domain (M2).[7-9]

The structure of muscle type AChRs from *Torpedo* electric organ has been determined to a resolution of 9 Å by Nigel Unwin's studies of two dimensional crystalline arrays of AChRs in membrane fragments.[27] This analysis reveals in side view an 80-Å-wide 120-Å-long protein extending 60 Å on the extracellular surface and 20 Å on the cytoplasmic surface. Viewed from the top, a pentagonal array of subunits is seen to surround a channel of 25 Å in diameter at its mouth which narrows abruptly at the level of the lipid bilayer and then flares open again on the cytoplasmic surface. Only a single transmembrane α helix was observed in each subunit. It was suggested that this α helix might correspond to the channel lining domain M2, but other evidence indicates that at least part of M2 is in a β conformation.[28] In any case, it seems that most of the transmembrane sequences of AChR subunits must be in β rather than α helical conformations. Because of the basic homologies in sequence between the subunits of all AChR subunits, it seems likely that they all have a basically similar size and pentagonal shape.

NEURONAL AChRs THAT DO NOT BIND α-BUNGAROTOXIN

Putative neuronal AChR subunit cDNAs have been identified by low stringency hybridization starting with muscle AChR probes.[9,29,30] These have been termed[29] α2–α6 if they contained a cysteine pair homologous to the pair at 192 and 193 in α1 subunits which can be affinity-labeled by ACh analogues.[8] Homologous cDNAs that did not contain this cysteine pair were termed β2–β4. Pairwise combinations of α2, α3, or α4 with β2 or β4 subunits form ACh-gated cation channels when coexpressed in *Xenopus* oocytes.[9,29,30] This allows for many potential AChR subtypes to be expressed in various regions or at different times during development. Although the localizations, developmental, and functional roles of these subtypes have not been worked out in detail, some simplifying generalizations have emerged. In the mature mammalian brain the predominant AChR subtype that does not bind αBgt is composed of α4 and β2 subunits.[31,32] These α4β2 AChRs comprise at least 90% of the high-affinity nicotine binding sites. In retina[33] and autonomic ganglia,[11] the predominant AChR subtype which does not bind αBgt includes α3 subunits.

AChRs with high affinity for nicotine have been immunoaffinity-purified from brains of chickens,[34,35] rats,[31] cattle, and humans[32] using antibodies. Such AChRs have subsequently been affinity-purified from rat brains by affinity chromatography using bromoacetylcholine-agarose.[36,37] In rats and chickens N-terminal amino acid sequencing identified α4 and β2 subunits as the components of this AChR subtype.[33,38,39] This conclusion has subsequently been confirmed by immunological analysis as well, using subunit-specific mAbs[32] and antipeptide sera.[33,40] Whereas in mammals[32] α4β2 AChRs comprise ≥90% of the brain AChRs with high affinity for nicotine, in chickens nearly half of such AChRs are formed by β2 in combination with an α subunit yet to be identified.[35] Evidence shows that a small fraction of α4β2 AChRs may have α5 subunits associated.[41] Mouse fibroblasts permanently transfected with α4 and β2 subunits exhibit ACh-gated cation channels and the same

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

pharmacological properties as native brain AChRs for binding of [^{3}H]nicotine and competing ligands.[42,43] The subunit stoichiometry was shown to be $(\alpha4)_2(\beta2)_3$ using a method that involved purifying metabolically labeled $\alpha4\beta2$ AChRs.[44] This method involved expressing these cDNAs in [^{35}S]methionine-labeled *Xenopus* oocytes, isolating fully assembled AChRs of the same ~10S size as native AChRs by sucrose gradient sedimentation, affinity-purifying these AChRs, purifying their subunits by PAGE, determining the ratio of [^{35}S]methionine label in the subunits, and then correcting for the methionine composition of these subunits.[44] This general method for stoichiometry determination was also shown to work for muscle AChR, and should work for any cloned multisubunit receptor similarly expressed. The same $(\alpha4)_2(\beta2)_3$ stoichiometry was also inferred from an electrophysiological method.[45] Thus, in the one case where the stoichiometry of a neuronal AChR subtype is known, the pentagonal symmetry of $(\alpha1)_2\beta1\gamma\delta$ AChRs of muscle is conserved, as presumably is the presence of two ACh binding sites; however, only two kinds of subunits compose that neuronal AChR subtype rather than the four kinds of subunits which compose muscle type AChRs.

Immunohistological localization of β2 subunits throughout rat brain using a subunit-specific mAb revealed a wide distribution[46] which closely paralleled that of high-affinity sites for binding [^{3}H]ACh or [^{3}H]nicotine, and which overlapped but was distinct from the pattern of [^{125}I]αBgt binding.[47] These results have subsequently been confirmed by others using an antipeptide serum to β2 antibodies.[48] Monoclonal antibodies have also been used to localize β2 subunits in chick brain and retina.[49–52] Transport of AChRs down the axons of retinal ganglion cells to their termination in the superior colliculus and other nuclei was demonstrated by showing that removal of an eye eliminated all labeling by a β2-specific mAb of the contralateral superior colliculus.[46] β2 was also located in dorsal root ganglion cells.[46] These results are consistent with the idea that many $\alpha4\beta2$ AChRs are located presynaptically where they may function to modulate the release of ACh or another transmitter. This is consistent with evidence for transport of AChRs in the habenulointerpeduncular tract,[51] and with evidence that AChRs can modulate the release of dopamine and other transmitters from synaptosomes.[53,54]

The $\alpha4\beta2$ AChRs account for > 90% of the high-affinity nicotine binding sites in mammalian brains,[32] and the amount of $\alpha4\beta2$ AChRs in brain is increased by chronic exposure to nicotine.[40] This effect does not appear to result from an increase in transcription of α4 or β2 subunits.[55] Instead, this effect appears to result from a decrease in the rate of destruction of $\alpha4\beta2$ AChRs.[56] This was demonstrated by showing that chronic exposure to 1 μM nicotine caused a twofold increase in the amount of $\alpha4\beta2$ AChRs in transfected fibroblasts, and that after prevention of synthesis of new proteins by cyclohexamide the AChRs already in the membrane were destroyed much less rapidly in the presence of nicotine.

The α3 AChRs are much more abundant in chick retina than are $\alpha4\beta2$ AChRs.[33] Chick ciliary ganglia have been shown, using subunit-specific antibodies, to contain AChRs which include the α3 subunit.[11] The α3 AChRs in ciliary ganglia appear to also contain β4 subunits and some may contain β2 subunits.[57] These AChRs also contain α5 subunits,[41] and are usually identified using an mAb to the main immunogenic region on α1 subunits,[58] which also crossreacts with a similar sequence on α5 subunits.[41] Human α5 subunits will assemble with human α3 and β2[59] or β4 subunits and be expressed on the surface of *Xenopus* oocytes, but α5 is not expressed on the surface when expressed alone or in combination with β2 subunits.[60] The exact subunit composition or stoichiometry of native α3 AChRs is not known, but it is clear that they do not include α7 subunits which are expressed in the same neurons.[57] The α3 AChRs have been immunohistologically located to the postsynaptic regions of the

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

cholinergic synapses on these neurons,[61,62] whereas the α7 AChRs have been localized away from these synapses on pseudodendrites[63] where the source of ACh to stimulate them is not obvious. However, it is not clear whether α3 AChRs will always be found at postsynaptic localizations or whether α7 AChRs will always be found at extrasynaptic locations.

NEURONAL AChRs THAT BIND α-BUNGAROTOXIN

α-Bungarotoxin binding proteins affinity-purified from chick brain yielded a partial amino acid sequence that[64] was used to design an oligonucleotide probe that was used to identify a cDNA for α7 subunits.[12] This cDNA was used to identify an α8 cDNA from chick brain which exhibited a very similar sequence.[12] α7 has subsequently also been cloned from rats[65] and humans.[15,16,66] α7 and α8 are homologous in sequence to, but about equally distinct from, muscle α1 and neuronal α2–α6.[12]

Demonstration that α7 and α8 are components of αBgt-binding AChRs depended on using mAbs to bacterially expressed large cytoplasmic domain fragments of α7 and α8 to immunoprecipitate [^{125}I]αBgt-labeled AChRs.[12] The epitopes for these mAbs have been mapped more precisely using synthetic α7 and α8 peptides.[67] Synthetic peptides have also been used to show that αBgt binds to amino acids within the sequence 180–200 of α7 and α8 subunits.[68] Most brain AChRs that could bind αBgt (75%) were found to contain α7 subunits, whereas a minority were found to contain both α7 and α8 subunits.[12] By contrast, in retina the majority of AChRs that can bind αBgt (69%) contain α8 subunits, and both α7α8 AChRs (17%) and α7 AChRs (14%) comprise minority populations.[69] The complete subunit composition or stoichiometry of these α7, α8 or α7α8 AChRs is not known, but it is clear from immunoprecipitation experiments that they do not contain other known AChR subunits. All preparations of purified αBgt binding neuronal AChRs that have been reported contain several peptide components,[31,70,71] and it is likely that some of these peptides correspond to AChR subunits that have yet to be identified.

Ballivet and co-workers first showed that α7 had the remarkably useful property of efficiently forming homo-oligomeric ACh-gated cation channels when expressed in *Xenopus* oocytes.[72] FIGURE 2 shows that α7 homomers are expressed as efficiently on the surface of *Xenopus* oocytes as are the native AChR subunit combinations α4β2 and α1β1γδ. FIGURE 3 shows that α7 homomers form ACh-gated cation channels, although the currents detected per α subunit are lower than for α4β2 AChRs or α1β1γδ AChRs. As will be discussed below, this is due in part to rapid desensitization of the α7 homomers. These three AChR subtypes differ in pharmacological properties, as illustrated in FIGURE 4. The α7 homomers have higher affinity for nicotine than ACh, whereas for α1β1γδ AChRs the opposite is true. Nicotine and ACh are most potent at activating the α4β2 AChR subtype, and they also have, by far, the highest equilibrium binding affinities for the desensitized conformation of this subtype.[32,42,75] Nicotine is a full agonist on α7 homomers and α4β2 AChRs, but only a partial agonist on α1β1γδ AChRs. All three subtypes exhibited Hill coefficients between 1.5 and 1.8. This suggests that not only for $(\alpha1)_2\beta1\gamma\delta$ AChRs (as has long been known),[76] but also for $(\alpha4)_2(\beta2)_3$ AChRs and even $(\alpha7)_5$ homomers, ACh must bind at two sites to provide sufficient energy to activate opening of the channel. This presumably reflects the basic homologies in the structures of these subtypes. Presumably only two binding sites are present in the subtypes with two α subunits, whereas activation of only two of five potential subunits is sufficient to activate α7 homomers.

The existence of efficiently expressed functional homomers of α7 greatly simplifies mutagenesis and expression studies, and has led to a series of very instructive

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

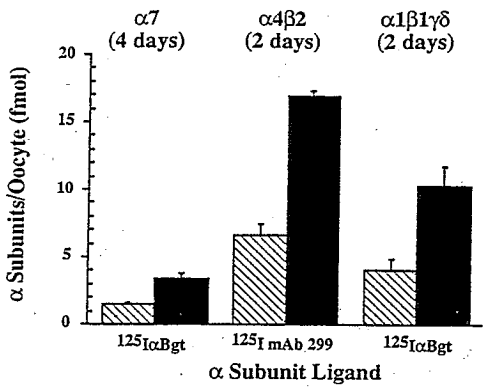

FIGURE 2. Comparison of surface ▨ and total ■ expression in *Xenopus* oocytes of chicken α7 homomers, chicken α4β2 AChRs, and *Torpedo* α1β1γδ AChRs. Note that the fraction of AChR on the surface is very similar for both α7 homomers (40%), α4β2 AChRs (35%), and α1β1γδ AChRs (38%). Oocytes were injected with 15 ng of cRNA for the indicated subunits and assayed 3 days later in this and all subsequent figures. Surface α7 and α1β1γδ AChRs on intact oocytes were measured using [^{125}I]αBgt. Surface α4β2 AChRs were measured using [^{125}I]mAb 299 to α4 subunits.[32] Total AChR concentrations were measured with the same [^{125}I]ligands using immunoisolated, detergent solubilized AChRs. Triton X-100 extracts of the oocytes were applied to Immulon-4 microwells coated with mAb 318 to α7 subunits,[73] or mAb 270 to β2 subunits,[35] or mAb 210 to α1 subunits.[74]

experiments by Changeux and co-workers. These include the demonstration that ion channel selectivity could be changed from cation to anion by mutagenesis of as few as two amino acids in the M2 region of α7 to correspond to amino acids found in homologous positions of glycine and GABA receptors,[77] and the demonstration that replacing the N-terminal domain of a 5HT-3 receptor with that of α7 produced ACh-gated channels characteristic of 5HT-3 receptors.[78] These experiments strikingly illustrate the fundamental homologies in structure between the different members of the ligand-gated ion channel superfamily which includes AChRs.

The α7 AChRs and α8 AChRs differ in their pharmacological properties.[69,73,79] The α7 AChRs have lower affinity for αBgt (K_d = 2 nM) than does muscle AChR, which binds αBgt nearly irreversibly, but α7 AChRs have higher affinity for αBgt than do α8 AChRs (K_d = 20 nM).[69,73] Because of their low affinity for αBgt, α8 AChRs may not be detected by the 1 nM concentrations of αBgt frequently used in binding studies. The α7 AChRs have much lower affinity for small cholinergic ligands than do α8 AChRs.[73] Also, α8 AChRs exhibit heterogeneity in ligand binding not seen with α7 AChRs. For example, the IC_{50} value for nicotine is 1.3 μM for α7 AChRs, 0.012 μM for 78% of the α8 AChR [^{125}I]αBgt binding sites, and 11 μM for 22% of the α8 AChR binding sites.[73] The heterogeneity in α8 AChR binding affinity may suggest that α8 associates with more than one kind of structural subunit. The heterogeneity could occur between two different ACh binding sites in one α8 AChR protein. For example, in a muscle AChR the affinity for curare of the ACh binding site formed by α1 and γ differs from that formed by α1 and δ.[24] Alternatively, two α8 AChR subtypes may occur which differ in subunit composition. Another interesting feature of the pharmacology of α7 AChRs and α8 AChRs is their relatively high affinity for the classic glycinergic antagonist strychnine and the classic muscarinic antagonist atro-

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

pine. The classic nicotinic antagonist curare has an IC_{50} = 7 μM for α7 AChRs and IC_{50} values of 0.79 μM and 65 μM for the two α8 AChR sites. Strychnine is nearly as potent, with an IC_{50} of 10 μM for α7 AChRs and IC_{50} values of 2 μM and 18 μM for the two α8 AChR sites. Atropine has an IC_{50} for α7 AChRs of 160 μM, but 0.031 μM and 390 μM for the two α8 AChR sites.

Pharmacological properties of α7 and α8 AChRs are generally, but not precisely, reflected by the properties of α7 and α8 homomers expressed in *Xenopus* oocytes.[79,80] The differences may be due to differences in posttranslational modifications in the expression system, but are more likely to be due to the lack of structural subunits normally associated with α7 and α8 subunits *in vivo*. The α7 homomers are expressed on the surface of oocytes at about the same 40% of total rate observed with α1β1γδ AChRs or α4β2 AChRs expressed in oocytes (FIG. 2), whereas <5% of α8 homomers are expressed on the surface at similar cRNA doses (15 ng).[79] At very high cRNA doses (100 ng), α8 homomer expression on the surface can increase to nearly 15%.[79] Separation of homomers on a sucrose gradient reveals that only α7 homomers of the ~10S size of native α7 AChRs can bind [^{125}I]αBgt,[80] whereas α8 homomers in various states of aggregation can bind [^{125}I]αBgt.[79] The α7 homomers immunoisolated on mAb-coated microwells had monotonic ligand binding properties,[80] but α8 homomers under these conditions displayed very broad binding curves.[79] This may reflect the observation in muscle AChRs that the ACh binding sites are formed at the interfaces between subunits.[8] Thus, ligands may only bind when adjacent α7 subunits are properly positioned in a fully assembled pentamer, whereas various associations

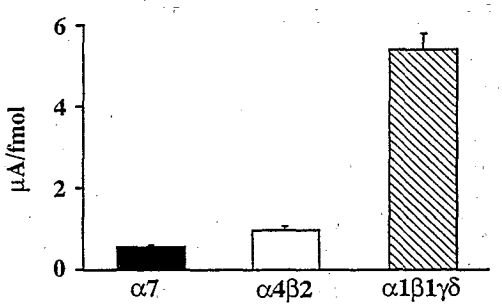

FIGURE 3. Comparison of current per surface α subunit for expression in *Xenopus* oocytes of chicken α7 homomers, chicken α4β2 AChRs, and *Torpedo* α1β1γδ AChRs, using saturating ACh at −90 mV. The experiments shown here and in subsequent figures used inhibitors of the Cl^- channel to prevent enhanced currents due to activation of the Cl^- channel by Ca^{2+} entering through the AChR channels. Note that the current/α subunit is much lower for α7 homomers than for the other AChR subtypes. As may become evident from subsequent figures, this was probably due to a combination of several factors including (1) rapid desensitization of α7, (2) possible side effects of the inhibitors of Cl^- channels used, and (3) the presence of five α subunits in an α7 homomer rather than two in an $(\alpha4)_2(\beta2)_3$ AChR or an $(\alpha1)_2\beta1\gamma\delta$ AChR. Surface α subunits were measured as shown in FIGURE 2. In this and all subsequent figures a two-electrode voltage clamp (Oocyte Clamp OC-725, Warner Instrument Corporation) was used as described below. The chamber was continuously perfused with 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, and 5 mM HEPES buffer pH 7.5 at 10 mL/min. The perfusing solution contained 0.5 μM atropine to block muscarinic receptors. The perfusing solution for α7 homomers and α4β2 AChRs also contained 100 μM each of niflumic and flufenamic acids to block Ca^{2+}-activated Cl^- channels. These were not added to α1β1γδ AChRs because their Ca^{2+} conductance was quite low.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

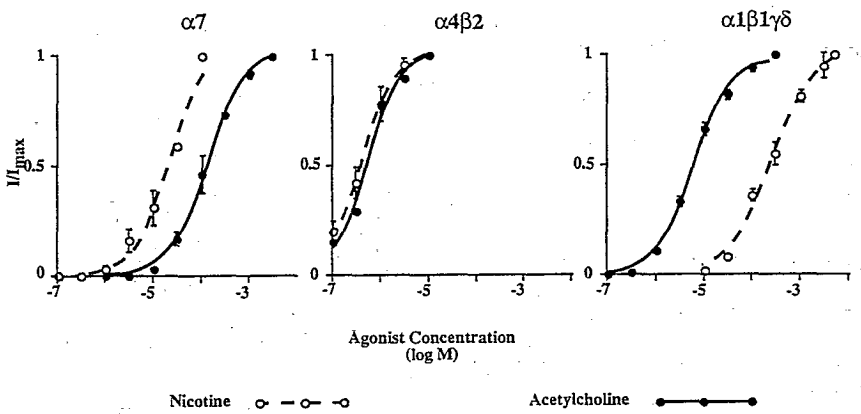

FIGURE 4. Comparison of ACh and nicotine dose/response curves for chicken α7 homomers, chicken α4β2 AChRs, and *Torpedo* α1β1γδ AChRs expressed in *Xenopus* oocytes. Note the contrasting pharmacological properties of the three AChR subtypes: (1) α7 homomers, like native α7 AChRs,[73] have higher affinity for nicotine (EC_{50} = 9 μM) than ACh (EC_{50} = 110 μM); (2) nicotine (EC_{50} = 0.42 μM) and ACh (EC_{50} = 0.45 μM) have equally high affinity for α4β2 AChRs; and (3) muscle-type AChRs have higher affinity for ACh (EC_{50} = 5.9 μM) than nicotine (EC_{50} = 245 μM). Saturating concentrations of both ACh and nicotine induced similar maximum currents with both α7 homomers and α4β2 AChRs. However, nicotine behaved as a partial agonist on α1β1γδ AChRs and, at saturating concentrations, produced currents 2–3-fold less than produced by saturating concentrations of ACh. Hill coefficients calculated at low concentrations of agonists (to avoid a reduction due to rapid desensitization at high concentrations) ranged between 1.5 and 1.8 in all cases. This suggests that not only for $(\alpha 1)_2\beta 1\gamma\delta$ AChRs[76] and $(\alpha 4)_2(\beta 2)_3$ AChRs, but also for $(\alpha 7)_5$ homomers, ACh acting at two sites is required to provide sufficient binding energy to activate opening of the channel.

of α8 subunits bind αBgt, but with differing affinities. Surface α8 homomers assayed for pharmacological effects on function exhibit monotonic dose/response curves,[79] suggesting that only properly assembled pentamers may be expressed on the surface. The limited sequence differences between the N-terminal extracellular domains of α7 and α8 subunits[12] presumably account for their pharmacological differences. Expression of a series of mosaics between α7 and α8 subunits reveal that virtually all of the pharmacological differences between α7 and α8 can be accounted for by amino acids between 179 and 208.[81]

The ion channel properties of α7 and α8 homomers are identical, reflecting their virtually identical sequences in the M1—M3 region.[79] As initially noted by Patrick and co-workers,[65] the most striking feature of these channels is that they are at least as selective for Ca^{2+} as are NMDA receptor channels. The increased Ca^{2+} selectivity of neuronal AChRs and α7 homomers, in particular, as compared to muscle type AChRs is illustrated in FIGURE 5. The Ca^{2+} that enters the channels can act as a second messenger and in *Xenopus* oocytes activates a Cl^- channel.[66,79] *In vivo,* this property could permit these AChRs to regulate many channels and processes, for example, neurite extension.[83–85] In *Xenopus* oocytes, activation of the Cl^- channel can be used to amplify the weak signal from α8 homomers. Another striking feature of α7 and α8 homomer channels is the rapidity with which the response desensitizes. Native α7 AChRs have been reported to desensitize so rapidly that especially fast

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

agonist application and fast electronics are required to measure channel opening accurately.[86–88] This no doubt has been responsible for the failure to detect the activity of these AChRs until quite recently. In oocytes, the relative slowness with which the large cells can be perfused may prolong the response and be used to experimental advantage. The rapid rate of α7 homomer desensitization as compared to α4β2 AChR or α1β1γδ AChR desensitization is shown in FIGURE 6. A third feature of their channels is strong inward rectification. Thus, as the cell depolarizes, α7 and α8 homomer channels close. This is the opposite of the rectification exhibited by NMDA receptors. Neuronal α4β2 AChRs, like α7 homomers, exhibit rectification, by contrast with muscle type AChRs, as shown in FIGURE 7. Curiously, both the rapid desensitization and inward rectification combine to minimize sustained ion flux through these channels. These self-limiting responses seem especially curious when considering the case of α7 AChRs at extrasynaptic locations removed from obvious sources of ACh.

The α7 AChRs have been histologically localized by binding of [^{125}I]αBgt[47] and mAbs,[50.52.69.89] and by *in situ* hybridization.[65] In rat brain α7 is prominent, for example

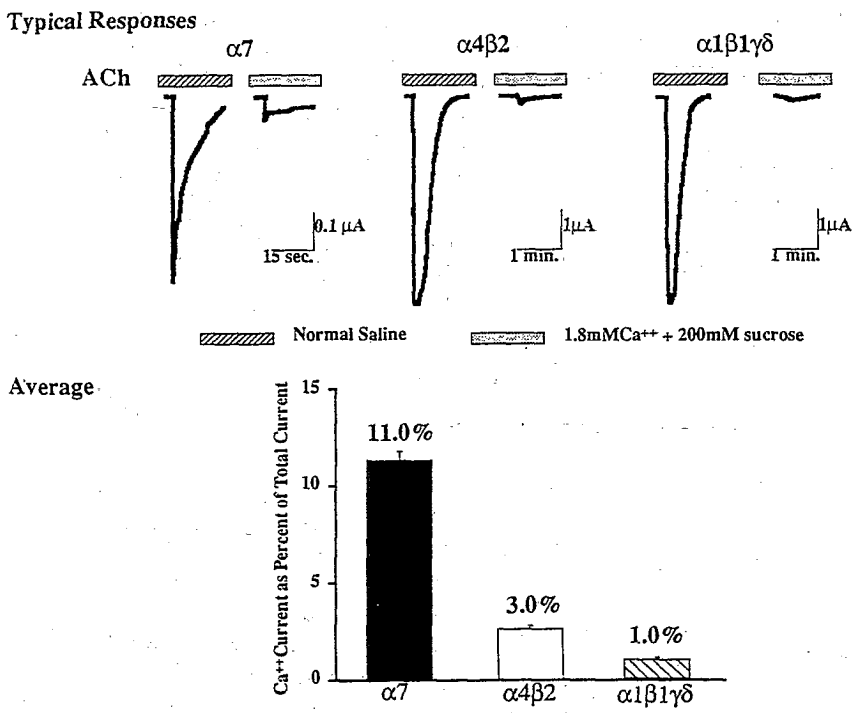

FIGURE 5. Comparison of the fraction of the current carried by Ca^{2+} for AChRs expressed in *Xenopus* oocytes by chicken α7 homomers, chicken α4β2 AChRs, and *Torpedo* α1β1γδ AChRs. Note that a much larger fraction of the total current through α7 homomers is carried by Ca^{2+} than is the case with the other AChR subtypes. This is consistent with other observations in oocytes and neurons.[65,82] All extracellular cations were replaced by 200 mM sucrose leaving only 1.8 mM $CaCl_2$ in the bathing solution.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

in the hippocampus.[65,89] This is a region not rich in α4β2 AChRs,[46] but rich in NMDA receptors. Albuquerque and co-workers have found α7-like AChR function to be present on nearly all hippocampal neurons in tissue culture.[86–88]

The α8 subunits have been immunohistologically localized in chick brain and retina.[50,52,69] α3, α7, α8, and β2 have also been immunohistologically localized in chick brain and retina.[33,49–52,69,90–92] Double-labeling studies have been employed to co-localize different subunits to particular cells.[91] Developmental studies in retina have traced the early development of neurons expressing α8 and α3 subunits starting from embryonic day 4.5.[92] Various types of amacrine and ganglion cells contain α3, β2, α7, and α8 subunits, whereas bipolar cells have only α8 subunits.[91] High-resolution localization of these AChRs in combination with electrophysiological studies will be necessary to understand their physiological roles.

Chick cochlear hair cells were shown by Fuchs and co-workers to exhibit a response to ACh from efferent endings of brain stem neurons which could be blocked by αBgt.[93,94] This response exhibits several interesting properties which might exemplify the sorts of synaptic mechanisms in which α7 and α8 AChRs may participate. It was found that ACh invoked Ca^{2+} influx through these AChRs which resulted in a long-lasting inhibitory hyperpolarizing response due to activation of Ca^{2+}-dependent K^+ channels. These AChRs could be blocked by αBgt, curare, atropine, and strychnine. We found that cochlear sensory epithelium contains α7 mRNA and have immunoisolated α7 but not α8 or α1 AChRs from cochlear sensory epithelium.[95] These immunoisolated cochlear AChRs display pharmacological properties identical to those of brain α7 AChRs. Newly identified α9 AChRs have also been detected in rat cochlear hair cells, and their pharmacology closely resembles that observed in chick cochlea.[96] Thus, hair cells express several AChR subunits.

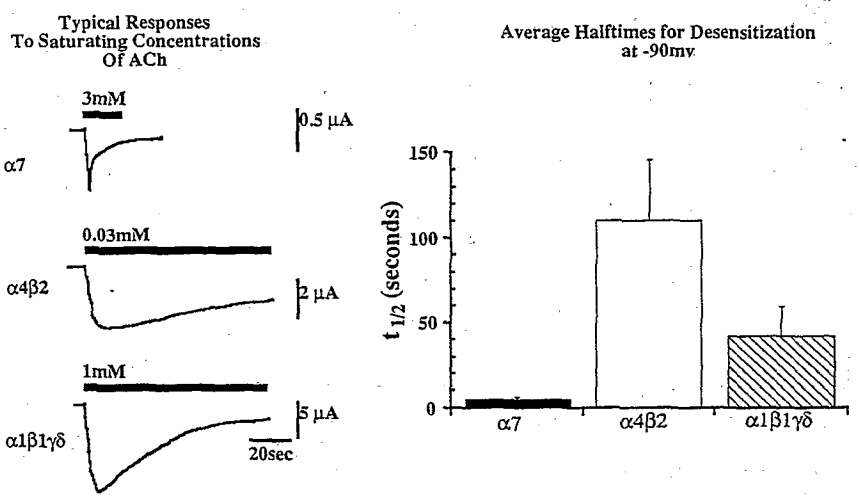

FIGURE 6. Comparison of rates of desensitization of chicken α7 homomers, chicken α4β2 AChRs, and *Torpedo* α1β1γδ AChRs expressed in *Xenopus* oocytes. Note that α7 homomers desensitize much more rapidly than the other AChR subtypes. The rate of desensitization may be underestimated due to the time necessary to perfuse the oocytes.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

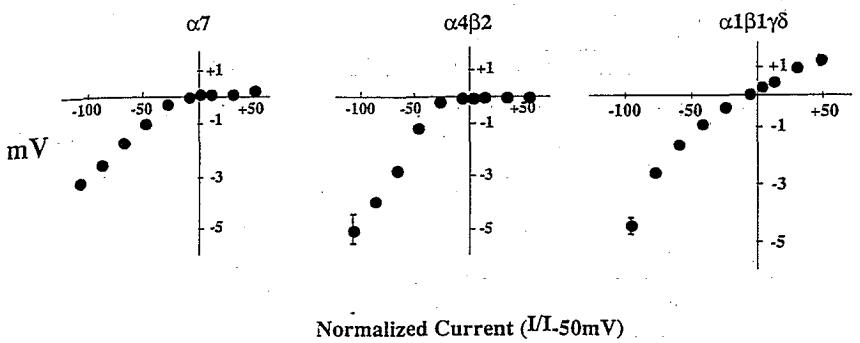

FIGURE 7. Comparison of rectification properties of chicken α7 homomers, chicken α4β2 AChRs, and *Torpedo* α1β1γδ AChRs expressed in *Xenopus* oocytes. Note that both neuronal α7 homomers and α4β2 AChRs exhibit strong inward rectification and thus close their channels at depolarizing potentials, whereas muscle-type AChRs exhibit a nonrectifying, relatively linear I/V curve.

CONCLUDING REMARKS

AChR subtypes from muscles and nerves exhibit structural and functional homologies dictated by the common evolutionary origin of their subunits, but they also differ significantly in important subtleties of their subunit compositions, pharmacological properties, ion channel properties, and functional roles. Muscle-type AChR is the best characterized ligand-gated ion channel, and is frequently used as a model for understanding both homologous and unrelated ligand-gated ion channels; however, as its structure is determined with increasing precision, surprises continue to be revealed. Much less is known about the structures and functions of neuronal AChRs, but it is likely that in addition to serving as the direct postsynaptic link in neurotransmission exemplified by muscle-type AChRs, some will also serve in different functional roles to which their structures have adapted.

REFERENCES

1. BETZ, H. 1990. Ligand-gated ion channels in the brain: The amino acid receptor superfamily. Neuron **5:** 383–392.
2. BARNARD, E. 1992. Receptor classes and the transmitter-gated ion channels. TIBS **17:** 368–374.
3. SEEBURG, P. 1993. The molecular biology of mammalian glutamate receptor channels. Trends Neurosci. **16:** 359–364.
4. LINDSTROM, J., G. SHELTON & Y. FUJII. 1988. Myasthenia gravis. Adv. Immunol. **42:** 233–284.
5. WONACOTT, S., M. RUSSELL & I. STOLERMAN. 1990. Nicotine Psychopharmacology: Molecular, Cellular, and Behavioral Aspects. Oxford Science Publications, Oxford, UK.
6. PETO, R., A. LOPEZ, J. BOREHAM, M. THUN & C. HEATH. 1992. Mortality from tobacco in developed countries: Indirect estimation from national vital statistics. Lancet **339:** 1268–1278.
7. LINDSTROM, J. 1994. Nicotine acetylcholine receptors. *In* CRC Handbook of Receptors. Alan North, Ed.: 153–176. CRC Press. Boca Raton, FL.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

8. KARLIN, A. 1993. Structure of nicotinic acetylcholine receptors. Curr. Opin. Neurobiol. **3:** 299–309.
9. SARGENT, P. 1993. The diversity of neuronal nicotinic acetylcholine receptors. Annu. Rev. Neurosci. **16:** 403–443.
10. DAS, M. & J. LINDSTROM. 1991. Epitope mapping of antibodies to acetylcholine receptors. Biochemistry **30:** 2470–2477.
11. SCHOEPFER, R., S. HALVORSEN, W. CONROY, P. WHITING & J. LINDSTROM. 1989. Antisera against an α3 fusion protein bind to ganglion but not to brain nicotinic acetylcholine receptors. FEBS Lett. **257:** 393–399.
12. SCHOEPFER, R., W. CONROY, P. WHITING, M. GORE & J. LINDSTROM. 1990. Brain α-bungarotoxin-binding protein cDNAs and mAbs reveal subtypes of this branch of the ligand-gated ion channel superfamily. Neuron **5:** 35–48.
13. ANAND, R., L. BASON, M. SAEDI, V. GERZANICH, X. PENG & J. LINDSTROM. 1993. Reporter epitopes: A novel approach to examine transmembrane topology of integral membrane proteins applied to the α1 subunit of the nicotinic acetylcholine receptor. Biochemistry **32:** 9975–9984.
14. ANAND, R. & J. LINDSTROM. 1992. Chromosomal localization of seven neuronal nicotinic receptor subunit genes in humans. Genomics **13:** 962–967.
15. CHINI, B., E. RAIMOND, A. ELGOYHEN, D. MORALLI, M. BALZARETTI & S. HEINEMANN. 1994. Molecular cloning and chromosomal localization of the human α7 nicotinic receptor subunit gene (CHRNA7). Genomics **19:** 379–381.
16. DONCETTESTAMM, L., L. MONTEGGIA, D. DONNELLY ROBERTS, M. WONG, J. LEE, J. TIAN & T. GIORDANO. 1993. Cloning and sequence of the human α7 nicotinic acetylcholine receptor. Drug Dev. Res. **30:** 252–256.
17. LOBOS, E., C. RUDNICK, M. WATSON & K. ISENBERG. 1989. Linkage disequilibrium study of RFLPs detected at the human muscle nicotinic acetylcholine receptor subunit genes. Am. J. Hum. Genet. **44:** 522–533.
18. BEESON, D., S. JEREMIAH, L. WEST, S. POVEY & J. NEWSOME-DAVIS. 1990. Assignment of the human nicotinic acetylcholine receptor genes: The α and δ subunit genes to chromosome 2 and the β subunit gene to chromosome 17. Ann. Hum. Genet. **54:** 199–208.
19. ENG, C., C. KOZAK, A. BEADET & H. ZOGHBI. 1991. Mapping of multiple subunits of the neuronal nicotinic acetylcholine receptor to chromosome 15 in man and chromosome 9 in mouse. Genomics **9:** 278–282.
20. MERLIE, J., R. SEBBANE, S. GARDNER, E. OLSON & J. LINDSTROM. 1983. The regulation of acetylcholine receptor expression in mammalian muscle. Cold Spring Harbor Symposia on Quantitative Biology **158:** 135–146.
21. TZARTOS, S., M. CUNG, P. DEMANGE, H. LOUTRARI, A. MAMALAKI, M. MARRAUD, I. PAPADOULI, C. SAKARELLOS & V. TSIKARIS. 1991. The main immunogenic region (MIR) of the nicotinic acetylcholine receptor and the anti-MIR antibodies. Mol. Neurobiol. **5:** 1–29.
22. SAEDI, M., R. ANAND, W. CONROY & J. LINDSTROM. 1990. Determination of amino acids critical to the main immunogenic region of intact acetylcholine receptors by in vitro mutagenesis. FEBS Lett. **267:** 55–59.
23. BLOUNT, P. & J. MERLIE. 1990. Mutational analysis of muscle nicotinic acetylcholine receptor subunit assembly. J. Cell Biol. **111:** 2612–2622.
24. BLOUNT, P. & J. MERLIE. 1989. Molecular basis of the two nonequivalent ligand binding sites of the muscle nicotinic acetylcholine receptor. Neuron **3:** 349–357.
25. VERRALL, S. & Z. HALL. 1992. The N-terminal domains of acetylcholine receptor subunits contain recognition signals for the initial steps of receptor assembly. Cell **68:** 23–31.
26. SAEDI, M., W. CONROY & J. LINDSTROM. 1991. Assembly of *Torpedo* acetylcholine receptor in *Xenopus* oocytes. J. Cell Biol. **112:** 1007–1015.
27. UNWIN, N. 1993. Nicotinic acetylcholine receptor at 9Å resolution. J. Mol. Biol. **229:** 1101–1124.
28. AKABAS, M., D. STAUFFER, M. XU & A. KARLIN. 1992. Acetylcholine receptor channel structure probed in cysteine-substitution mutants. Science **258:** 307–310.
29. DENERIS, E., J. CONNOLLY, S. ROGERS & R. DUVOISIN. 1991. Pharmacological and

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

functional diversity of neuronal nicotinic acetylcholine receptors. Trends Pharmacol. Sci. **12:** 34–40.

30. HEINEMANN, S., J. BOULTER, J. CONNOLLY, E. DENERIS, R. DUVOISIN, M. HARTLEY, I. HERMANS-BORGMEYER, M. HOLLMANN, A. O'SHEA-GREENFIELD, A. PAPKE, S. ROGERS & J. PATRICK. 1991. The nicotinic receptor genes. Clin. Neuropharmacol. **14:** S45–S61.
31. WHITING, P. & J. LINDSTROM. 1987. Purification and characterization of a nicotinic acetylcholine receptor from rat brain. Proc. Natl. Acad. Sci. USA **84:** 595–599.
32. WHITING, P. & J. LINDSTROM. 1988. Characterization of bovine and human neuronal nicotinic acetylcholine receptors using monoclonal antibodies. J. Neurosci. **8:** 3395–3404.
33. WHITING, P., R. SCHOEPFER, W. CONROY, M. GORE, K. KEYSER, S. SHIMASAKI, F. ESCH & J. LINDSTROM. 1991. Differential expression of nicotinic acetylcholine receptor subtypes in brain and retina. Mol. Brain Res. **10:** 61–70.
34. WHITING, P. & J. LINDSTROM. 1986. Purification and characterization of a nicotinic acetylcholine receptor from chick brain. Biochemistry **25:** 2082–2093.
35. WHITING, P., R. LIU, B. MORLEY & J. LINDSTROM. 1987. Structurally different neuronal nicotinic acetylcholine receptor subtypes purified and characterized using monoclonal antibodies. J. Neurosci. **7:** 4005–4016.
36. NAKAYAMA, H., M. SHIRASE, T. NAKASHIMA, Y. KUROGOCHI & J. LINDSTROM. 1990. Affinity purification of nicotinic acetylcholine receptor from rat brain. Mol. Brain Res. **7:** 221–226.
37. NAKAYAMA, H., H. OKUDA & T. NAKASHIMA. 1993. Phosphorylation of rat brain nicotinic acetylcholine receptor by cAMP-dependent protein kinase in vitro. Mol. Brain Res. **20:** 171–177.
38. WHITING, P., F. ESCH, S. SHIASAKI & J. LINDSTROM. 1987. Neuronal nicotinic acetylcholine receptor β subunit is coded for by the cDNA clone α4. FEBS Lett. **219:** 459–463.
39. SCHOEPFER, R., P. WHITING. F. ESCH, R. BLACHER, S. SHIMASAKI & J. LINDSTROM. 1988. cDNA clones coding for the structural subunit of a chicken brain nicotinic acetylcholine receptor. Neuron **1:** 241–248.
40. FLORES, C., S. ROGERS, L. PABREZA, B. WOLFE & K. KELLAR. 1992. A subtype of nicotinic cholinergic receptor in rat brain is composed of α4 and β2 subunits and is upregulated by chronic nicotine treatment. Mol. Pharmacol. **41:** 31–37.
41. CONROY, W., A. VERNALLIS & D. BERG. 1992. The α5 gene product assembles with multiple acetylcholine receptor subunits to form distinctive receptor subtypes in brain. Neuron **9:** 1–20.
42. WHITING, P., R. SCHOEPFER, J. LINDSTROM & T. PRIESTLY. 1991. Structural and pharmacological characterization of the major brain nicotinic acetylcholine receptor subtype stably expressed in mouse fibroblasts. Mol. Pharmacol. **40:** 463–472.
43. PEREIRA, E., M. ALKONDON, S. REINHARDT-MAELICKE, X. PENG, J. LINDSTROM, P. WHITING & E. ALBUQUERQUE. 1994. Physostigmine and galanthamine reveal the presence of the novel binding site on the α4β2 subtype of neuronal nicotinic acetylcholine receptors stably expressed in fibroblast cells. J. Pharmacol. Exp. Ther. **270:** 768–778.
44. ANAND, R., W. CONROY, R. SCHOEPFER, P. WHITING & J. LINDSTROM. 1991. Chicken neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes have a pentameric quaternary structure. J. Biol. Chem. **266:** 11192–11198.
45. COOPER, E., S. COUTURIER & M. BALLIVET. 1991. Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor. Nature **350:** 235–238.
46. SWANSON, L., D. SIMMONS, P. WHITING & J. LINDSTROM. 1987. Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. J. Neurosci. **7:** 3334–3342.
47. CLARKE, P., R. SCHWARTZ, S. PAUL, C. PERT & A. PERT. 1985. Nicotinic binding in rat brain: Autoradiographic comparison of [^{3}H] nicotine, and [^{125}I] α-bungarotoxin. J. Neurosci. **5:** 1307–1315.
48. HILL, J., M. ZOLI, J. BOURGEOIS & J. CHANGEUX. 1993. Immunocytochemical localization of a neuronal nicotinic receptor: The β2 subunit. J. Neurosci. **13:** 1551–1568.
49. KEYSER, K., T. HUGHES, P. WHITING, J. LINDSTROM & H. KARTEN. 1988. Cholinoceptive

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

neurons in the retina of the chick: An immunohistochemical study of the nicotinic acetylcholine receptors. Visual Neurosci. **1:** 349–366.

50. BRITTO, L., D. HAMASSAKI-BRITTO, E. FERRO, K. KEYSER, H. KARTEN & J. LINDSTROM. 1992. Neurons of the chick brain and retina expressing both α-bungarotoxin-sensitive and α-bungarotoxin-insensitive nicotinic acetylcholine receptors: An immunohistochemical analysis. Brain Res. **590:** 193–200.
51. CLARKE, P., G. HAMILL, N. NADI, D. JACOBOWITZ & A. PERT. 1986. ^{3}H-Nicotine and ^{125}I-α-bungarotoxin-labeled nicotinic receptors in the interpeduncular nucleus of rats. II. Effects of habenular deafferentation. J. Comp. Neurol. **251:** 407–413.
52. BRITTO, L., K. KEYSER, J. LINDSTROM & H. KARTEN. 1992. Immunohistochemical localization of nicotinic acetylcholine receptor subunits in the mesencephalon and diencephalon of the chick (*Gallus gallus*). J. Comp. Neurol. **317:** 325–340.
53. RAPIER, C., G. LUNT & S. WONNACOTT. 1988. Stereoselective nicotine-induced release of dopamine from striatal synaptosomes: Concentration dependence and repetitive stimulation. J. Neurochem. **50:** 1123–1130.
54. GRADY, S., M. MARKS & A. COLLINS. 1994. Desensitization of nicotine-stimulated ^{3}H dopamine release from mouse striatal synaptosomes. J. Neurochem. **62:** 1390–1398.
55. MARKS, M., J. PAULY, D. GROSS, E. DENERIS, I. HERMANS-BORGMEYER, S. HEINMANN & A. COLLINS. 1992. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. J. Neurosci. **12:** 2765–2784.
56. PENG, X., V. GERZANICH, R. ANAND, P. WHITING & J. LINDSTROM. 1994. Nicotine-induced increase in neuronal nicotinic receptors results from a decrease in the rate of receptor turnover. Mol. Pharmacol. **46:** 523–530.
57. VERNALIS, A., W. CONROY & D. BERG. 1993. Neurons assemble acetylcholine receptors with as many as three kinds of subunits while maintaining subunit segregation among receptor subtypes. Neuron **10:** 451–464.
58. TZARTOS, S., D. RAND, B. EINARSON & J. LINDSTROM. 1981. Mapping of surface structures on *Electrophorus* acetylcholine receptor using monoclonal antibodies. J. Biol. Chem. **256:** 8635–8645.
59. ANAND, R. & J. LINDSTROM. 1990. Nucleotide sequence of the human nicotinic acetylcholine receptor β2 subunit gene. Nucleic Acids Res. **18:** 4272.
60. WANG, F. & J. LINDSTROM. Unpublished results.
61. JACOB, M., D. BERG & J. LINDSTROM. 1984. A shared antigenic determinant between the *Electrophorus* acetylcholine receptor and a synaptic component on chick ciliary ganglion neurons. Proc. Natl. Acad. Sci. USA **81:** 3223–3227.
62. JACOB, M., J. LINDSTROM & D. BERG. 1986. Surface and intracellular distribution of a putative neuronal nicotinic acetylcholine receptor. J. Cell Biol. **103:** 205–214.
63. JACOB, M. & D. BERG. 1983. The ultrastructural localization of α-bungarotoxin binding sites in relation to synapses on chick ciliary ganglion neurons. J. Neurosci. **3:** 260–271.
64. CONTI-TRONCONI, B., S. DUNN, E. BARNARD, J. DOLLY, F. LAI, N. RAY & M. RAFTERY. 1985. Brain and muscle nicotinic acetylcholine receptors are different but homologous proteins. Proc. Natl. Acad. Sci. USA **82:** 5208–5212.
65. SÉGUÉLA, P., J. WADICHE, K. DINELLY-MILLER, J. DANI & J. PATRICK. 1993. Molecular cloning, functional properties, and distribution of rat brain α7: A nicotinic cation channel highly permeable to calcium. J. Neurosci. **13:** 596–604.
66. PENG, X., M. KATZ, V. GERZANICH, R. ANAND & J. LINDSTROM. 1994. Human α7 acetylcholine receptor: Cloning of the α7 subunit from the SH-SY5Y cell line and determination of pharmacological properties of native receptors and functional α7 homomers expressed in *Xenopus* oocytes. Mol. Pharmacol. **45:** 546–554.
67. MCLANE, K., X. WU, J. LINDSTROM & B. CONTI-TRONCONI. 1992. Epitope mapping of polyclonal and monoclonal antibodies against two α-bungarotoxin binding subunits from neuronal nicotinic receptors. J. Neuroimmunol. **38:** 115–128.
68. MCLANE, K., X. WU, R. SCHOEPFER, J. LINDSTROM & B. CONTI-TRONCONI. 1991. Identification of sequence segments forming the α-bungarotoxin binding sites on two nicotinic acetylcholine receptor α subunits from the avian brain. J. Biol. Chem. **266:** 15230–15239.
69. KEYSER, K., L. BRITTO, R. SCHOEPFER, P. WHITING, J. COOPER, W. CONROY, A.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

BROZOZOWSKA-PRECHTL, H. KARTEN & J. LINDSTROM. 1993. Three subtypes of α-bungarotoxin sensitive nicotinic acetylcholine receptors are expressed in chick retina. J. Neurosci. **13:** 442–454.

70. GOTTI, C., A. OGANDO, W. HANKE, R. SCHULE, M. MORETTI & F. CLEMENTI. 1991. Purification and characterization of an α-bungarotoxin receptor that forms a functional nicotinic channel. Proc. Natl. Acad. Sci. USA **88:** 3258–3262.
71. GOTTI, C., M. MORETTI, R. LONGHI, L. BRISCINI, E. MANURA & F. CLEMENTI. 1993. Antipeptide specific antibodies for the characterization of different α subunits of α-bungarotoxin binding acetylcholine receptors present in chick optic lobe. J. Recept. Res. **13:** 453–465.
72. COURTURIER, S., D. BERTRAND, J. MATTER, M. HERNANDEZ, S. BERTRAND, N. MILLAR, S. VALERA, T. BARKAS & M. BALLIVET. 1990. A neuronal nicotinic acetylcholine receptor subunit (α7) is developmentally regulated and forms a homomeric channel blocked by α-bungarotoxin. Neuron **5:** 847–856.
73. ANAND, R., X. PENG, J. BALLESTA & J. LINDSTROM. 1993. Pharmacological characterization of α-bungarotoxin sensitive AChRs immunoisolated from chick retina: Contrasting properties of α7 and α8 subunit-containing subtypes. Mol. Pharmacol. **44:** 1046–1050.
74. CONROY, W., M. SAEDI & J. LINDSTROM. 1990. TE671 cells express an abundance of a partially mature acetylcholine receptor α subunit which has characteristics of an assembly intermediate. J. Biol. Chem. **265:** 21642–21651.
75. WHITING, P. & J. LINDSTROM. 1986. Pharmacological properties of immunoisolated neuronal nicotinic receptors. J. Neurosci. **6:** 3061–3069.
76. JACKSON, M. 1989. Perfection of a synaptic receptor: Kinetics and energetics of the acetylcholine receptor. Proc. Natl. Acad. Sci. USA **86:** 2199–2203.
77. GALZI, J., A. DEVILLERS-THIERY, N. HUSSY, S. BERTRAND, J. CHANGEUX & D. BERTRAND. 1992. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. Nature **359:** 500–505.
78. EISILE, J., S. BERTRAND, J. GALZI, A. DEVILLERS-THIERY, J. CHANGEUX & D. BERTRAND. 1993. Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. Nature **366:** 479–409.
79. GERZANICH, V., R. ANAND & J. LINDSTROM. 1994. Homomers of α8 subunits of nicotinic receptors functionally expressed in *Xenopus* oocytes exhibit similar channel but contrasting binding site properties compared to α7 homomers. Mol. Pharmacol. **45:** 212–220.
80. ANAND, R., X. PENG & J. LINDSTROM. 1993. Homomeric and native α7 acetylcholine receptors exhibit remarkably similar but nonidentical pharmacological properties suggesting that the native receptor is a heteromeric protein complex. FEBS Lett. **327:** 241–246.
81. ANAND, R. & J. LINDSTROM. Unpublished results.
82. VERNINO, S., M. AMADOR, C. LUETJE, J. PATRICK & J. DANI. 1992. Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. Neuron **8:** 127–134.
83. LIPTON, S., M. FROSH, M. PHILLIPS, D. TAUCK & E. AIZENMAN. 1988. Nicotinic antagonists enhance process outgrowth by rat retinal ganglion cells in culture. Science **239:** 1293–1296.
84. ZHANG, Z., S. VIJAYAROGHAVEN & D. BERG. 1994. Neuronal acetylcholine receptors that bind α-bungarotoxin with high affinity function as ligand-gated ion channels. Neuron **12:** 167–177.
85. PUGH, P. & D. BERG. 1994. Neuronal acetylcholine receptors that bind α-bungarotoxin mediate neurite retraction in a calcium-dependent manner. J. Neurosci. **14:** 889–896.
86. ALKONDON, M. & E. ALBUQUERQUE. 1991. Initial characterization of the nicotinic acetylcholine receptors in rat hippocampal neurons. J. Recept. Res. **11:** 1101–1201.
87. ALKONDON, M. & E. ALBUQUERQUE. 1993. Diversity of nicotinic acetylcholine receptors in rat hippocampal neurons. I. Pharmacological and functional evidence for distinct structural subtypes. J. Pharmacol. Exp. Ther. **265:** 1455–1473.
88. CASTRO, N. & E. ALBUQUERQUE. 1993. Brief-lifetime, fast-inactivating ion channels account for the α-bungarotoxin-sensitive nicotinic response in hippocampal neurons. Neurosci. Lett. **164:** 137–140.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000

89. DEL TORO, J. JUIZ, X. PENG, J. LINDSTROM & M. CRIADO. 1994. Immunocytochemical localization of the α7 subunit of the nicotinic acetylcholine receptor in the rat central nervous system. J. Comp. Neurol. **349:** 325–342.
90. HAMASSAKI-BRITTO, D., A. BRZOZOWSKA-PRECHTL, H. KARTEN, J. LINDSTROM & K. KEYSER. 1991. GABA-like immunoreactive cells containing nicotinic acetylcholine receptors in the chick retina. J. Comp. Neurol. **313:** 394–408.
91. HAMASSAKI-BRITTO, D., A. BRZOZOWSKA-PRECHTHL, H. KARTEN & J. LINDSTROM. 1994. Bipolar cells of the chick retina containing α-bungarotoxin-sensitive nicotinic acetylcholine receptors. Visual Neurosci. **11:** 63–70.
92. HAMASSAKI-BRITTO, D., P. GARDINO, J. HOKOC, K. KEYSER, H. KARTEN, J. LINDSTROM & L. BRITTO. 1994. Differential development of α-bungarotoxin-sensitive and α-bungarotoxin-insensitive nicotinic acetylcholine receptors in the chick retina. J. Comp. Neurol. **347:** 161–170.
93. FUCHS, P. & B. MURROW. 1992. Cholinergic inhibition of short (outer) hair cells of the chick's cochlea. J. Neurosci. **12:** 800–809.
94. FUCHS, P. & B. MURROW. 1992. A novel cholinergic receptor mediates inhibition of chick cochlear hair cells. Proc. R. Soc. Lond. B. **248:** 35–40.
95. ANAND, R., V. GERZANICH, J. COOPER, X. PENG, P. FUCHS & J. LINDSTROM. *Unpublished* results.
96. ELGOYHEN, A., D. JOHNSON, J. BOULTER, D. VETTER & S. HEINEMANN. 1994. α9: An acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. Cell **79:** 705–715.

Source: https://www.industrydocuments.ucsf.edu/docs/tqvb0000